Remarks

Claims 39-53 are currently pending.

Claims 39, 44 and 49 have been amended to recite that the part of the isolated nucleic acid fragment has to be sufficient in length for use in antisense inhibition or sense suppression. Support for this may be found in the specification on page 12 at lines 22-38, page 34 line 33 through 6 on page 35, and Example 20. Thus, it is believed that no new matter has been added.

Claims 39-53 were rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement, because the specification purportedly fails to describe the structural features that are essential for LKR or LKR/SDH activity, and those sequences sufficient for use in antisense inhibition or sense suppression of LKR/SDH.

Appendix A (submitted herewith) depicts the first two steps of the alpha-aminoadipic acid pathway. This pathway is used in plants and animals to catabolize lysine, whereas yeast and fungi use the very same pathway to synthesize lysine. These two groups of organisms also possess structurally distinct forms of enzymes in this pathway, namely Lysine-ketoglutarate reductase (LKR) and saccharopine dehydrogenase (SDH). In plants and animals, these enzymes are linked to form a single bifunctional polypeptide (LKR/SDH), while in yeast and fungi they exist as separate entities (lysine-forming SDH and glutamate-forming SDH).

This is discussed in the specification on pages 31-35.

Attached hereto is Appendix B which is an alignment of the LKR domains of the plant bifunctional LKR/SDH proteins from Arabidopsis (SEQ ID NO:112), corn (SEQ ID NO:122, encoded by SEQ ID NO:120) and soybean (SEQ ID NO:121) and the monofunctional lysine-forming SDH proteins from *S.cerevisiae* (gi:453184), *C.albicans* (gi:1170847) and *Y.lipolytica* (gi:173262).

Appendix C (submitted herewith) is comparison of the SDH domains of the bifunctional plant LKR/SDH proteins from Arabidopsis (SEQ ID NO:112), corn (SEQ ID NO:122, encoded by SEQ ID NO:120) and soybean (SEQ ID NO:121) and the monofunctional glutamate-forming SDH protein from *S.cerevisiae* (gi:729968). Residues that are identical among at least one of the plant sequences and at least one of the yeast sequences are indicated by an asterix above each alignment.

Residues that are identical among at least two plant sequences are indicated by a plus sign above each alignment.

The plant LKR domains share about 70% and 60% sequence identity with each other, respectively, whereas the plant LKR domains and yeast lysine-forming SDH proteins share between 15% and 17% sequence identity.

The plant SDH domains share about 60% sequence identity among each other and around 30% sequence identity with the yeast protein. Alignments and percent identity calculations were performed using the Clustal V method of alignment.

The comparisons in Appendices B and C demonstrate the sequences of the invention possess stretches of highly conserved regions. One skilled in the art would appreciate that the more highly conserved a residue is, the less likely that it could be modified and function maintained. From these alignments, one could quickly determine which amino acid residues might be modified in SEQ ID NO:122 (encoded by SEQ ID NO:120) without a likely change in function.

In the instant specification, the cDNA fragments of the bifunctional Arabidopsis LKR/SDH were identified based on the homology to the monofunctional proteins from yeast. The sequence similarity between the yeast and plant polypeptides (Fig.9 of instant specification) demonstrated that these cDNAs encode Arabidopsis saccharopine dehydrogenase.

The complete genomic sequence of the Arabidopsis LKR/SDH gene was subsequently isolated and the cDNA sequence and corresponding amino acid sequence determined. The LKR/SDH cDNA revealed an ORF of 3.16 kb, which predicts a protein of 117 kd, and confirms that the LKR and SDH enzymes reside on one polypeptide.

In order to isolate further plant LKR/SDH sequences, degenerate primers based upon comparison of the Arabidopsis LKR/SDH amino acid sequence with that of other LKR proteins were designed. These were used to amplify soybean and corn LKR/SDH fragments using PCR from mRNA , or cDNA synthesized from mRNA, isolated from developing soybean or corn seeds. Near full length sequences for the LKR/SDH sequences were obtained using Race and hybridization protocols. Furthermore, partial rice and wheat were isolated based on homology to the Arabidopsis protein.

Appendix D (submitted herewith) is an alignment of the plant bifunctional LKR/SDH proteins from Arabidopsis (SEQ ID NO:112), corn (SEQ ID NO:122) and soybean (SEQ ID NO:121). Amino acid residues identical among at least two plant sequences are indicated by an asterisk on the top row; dashes are used by the program to maximize the alignment of the sequences. The LKR and SDH domains have been boxed in Appendix D to facilitate review of the enclosed Appendix D. It should also be noted that, in addition to the LKR and SDH domains, a high degree of homology is also observed in the intermediary or 'spacer' region of the bifunctional LKR-SDH polypeptide.

Attention is kindly invited to Tang et al. (*Plant Cell* 9:1305-1316 (1997), (copy submitted previously) and Epelbaum et al. (*Plant Mol. Biol.* 35:735-748 (1997), (copy submitted previously), which disclosed the *Arabidopsis* LKR-SDH sequence. The aforementioned publications discuss the LKR and SDH domains of the bifunctional protein that were identified by homology to the corresponding monofunctional proteins from yeast, and by expressing the LKR and SDH domains of the bifunctional LKR-SDH separately in bacteria or yeast. The expression studies showed that the separate LKR and SDH domains conferred the expected activity and specifictiy to the transformed cells.

Furthermore, as was described in Dr. Carl Falco's declaration, dated August 24th, 2000 (copy previously submitted), a 1268 bp gene fragment including the LKR coding domain of the corn LKR-SDH sequence (SEQ ID NO:122) was successfully used in cosuppression studies to produce seeds having increased accumulation of lysine. This increase in lysine appeared to be directly related to the cosuppression of LKR/SDH.

The corn LKR/SDH cDNA sequence was further used to identify transposon mutations in the endogenous corn LKR/SDH gene. The precise location of Mutator insertions into the LKR/SDH gene was determined by sequencing of genomic DNA from individual mutants. An insertion mutation located in an exon in the LKR domain of the gene was chosen for further study and it was confirmed that knocking out LKR/SDH, by itself, was able to increase seed lysine content in corn seeds.

Subsequently, this LKR/SDH mutator line was crossed to a transgenic line that accumulates excess free lysine due to expression of lysine insensitive versions of the enzymes involved in lysine biosynthesis, DHDPS and AK. A further increase in lysine

was observed in seeds that contained both modifications (feedback-insensitive versions of the biosynthetic enzymes and suppression of the enzymes involved in lysine catabolism).

In view of the above discussion, it is respectfully submitted that the written description requirement has been fulfilled. Withdrawal of this ground of rejection is respectfully requested.

Claims 39-53 were rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. Specifically, it is stated on page 4 of the Office Action that the "specification does not demonstrate that any of the claimed sequences have homology to saccharopine dehydrogenase (SDH) and that SEQ ID NO: 120 and 122 are not full length sequences (page 34). Therefore, it is even more uncertain that the claimed sequences would encode the portions required to confer LKR activity. "

It is respectfully submitted that the above discussion is equally apposite to this ground of rejection.

It is further stated in the Office Action that "De Luca teaches that modifying plant biosynthetic pathways by transforming plants with genes encoding enzymes involved in a biosynthetic pathway is highly unpredictable and often the desirable results are impossible to achieve".

It is respectfully submitted that ample information is available in case of the lysine biosynthetic and catabolic pathways that clearly demonstrates how to increase lysine production via modification of the biosynthetic and catabolic pathways. The use of lysine feedback-insensitive versions of the key biosynthetic enzymes, DHDPS and AK, has been shown to lead to an increase in free lysine levels. The instant specification teaches that blocking the first step in lysine catabolism will lead to increased accumulation of lysine. One of ordinary skill in the art would be able to practice the claimed invention without engaging in undue experimentation.

Accordingly, withdrawal of the rejection of the claims under 35 U.S.C. 112, first paragraph as lacking enablement is respectfully requested in view of the above discussion. It is respectfully submitted that the claims are now in form for allowance which allowance is respectfully requested.

A Petition for a three (3) month Extension of time accompanies this response. Please charge any fees or credit any overpayment which are associated with the filing of this Preliminary Amendment to Deposit Account No. 04-1928 (E. I. du Pont de Nemours and Company).

Respectfully submitted,

/Lynne M. Christenbury/

LYNNE M. CHRISTENBURY
ATTORNEY FOR APPLICANT
Registration No.: 30,971
Telephone: (302) 992-5481
Facsimile: (302) 892-1026

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